Acute and Sub-acute toxicity studies of a herbal formulation
Kadukkai Chooranam.

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ABSTRACT

Introduction
Kadukkai chooranam is an effective drug composition from siddha medicine used for the management of Menorrhagia/Dysfunctional Uterine bleeding (Pitha perumbaadu) which is referenced from a classical age-old text Agathiyar attavani vaagadam.

Aim of this study
The aim of study was to assess the acute and sub-acute toxicity of the polyherbal composition kadukkai chooranam.

Methodology:
In the acute toxicity study, a single dose of 2000 mg/kg was administered to wistar albino female rat (200-220g) after the proper acclimatization and fasting procedures was made as per the OECD 423 – acute toxicity guidelines. All the animals were observed for physical symptoms and behavioral changes for first 72 h. In sub-acute toxicity study repeated doses of the polyherbal composition was administered to Wistar albino rats of both genders, separately acclimatized for 7 days and fasted as per OECD –407 guidelines. The animals received two doses of drug (100 mg/kg/day and 200 mg/kg/day) for a period of 28 days. On 28th day of experiment, blood sampling of animals was done for hematological and biochemical analysis i.e. liver and renal function parameters, lipid profile and then sacrificed for histopathological study of liver and kidney. The rats were observed daily during the period of study, and sacrificed on the 29th day. Observation parameters of the animals included a comparative evaluation of general appearance/behaviour, morbidity/mortality, body weights, haematology, biochemistry, lipid profile and histopathology of major organs of treated and control groups.

Results
There was no morbidity and mortality noticed with single dose administration in acute toxicity study in wistar rats. In sub-acute toxicity study, no morphological changes were observed in kidney, liver and spleen of animals at dose of 100 mg/kg/day and 200 mg/kg/day.

Conclusions
The results of the present study show that oral administration of kadukkai chooranam did not produce any severe toxic effects in both acute and sub-acute studies in Wistar rats. Therefore, usage of an appropriate dosage will be preferable and considered as safe.

KEYWORDS
Acute toxicity, Kadukkai chooranam, Terminalia chebula, OECD
INTRODUCTION

A toxicological investigation is considered very essential for the development of new drugs. Plant based medicine i.e traditional medicine from time immemorial has been the mainstay of health care need for the treatment of various types of diseases. Despite improvement in science and technology in medicine, greater numbers of the population are still relying on herbal medicine to resolve their primary health problems. (Breeher et al., 2015)

In most developed countries, there appears to be increased awareness of the usefulness of herbal drugs in the management of various disease conditions. According to a World Health Organization estimate, more than 80% of the world’s population relies on traditional medicine for their primary healthcare needs. It is generally presumed that herbal medicines are more effective and because of their natural source are free from undesirable side effect.(Gunturu et al., 2011)

This belief has led to serious abuse such as prolonged administration without appropriate dose monitoring or without the supervision of physicians thereby undermining the greater potential for adverse effects. But in herbal medicine doesn’t provide that many highly measurable negative effects. (Prakash et al., 2009) The danger associated with the potential toxicity of herbal therapies administered over a long period of time demand that the practitioners be kept abreast of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs.

Siddha and ayurveda medicine is still practiced in India where approximately 85% of the Indian population uses crude plant extract/formulations for the treatment of various diseases. The traditional uses of plants may cause adverse effects in humans or animals. A number of plants and their constituents traditionally used as medicines are suspected of being carcinogens to rodents and/or humans. (Ying et al., 2018)

Further, there is a scarcity in scientific evidence on the safety and efficacy of herbal drugs on the increase in science and technology in medicine, greater numbers of the population are still relying on herbal medicine to resolve their primary health problems. (Breeher et al., 2015)

This drug mainly composed of Terminalia chebula fruit rind and leaves of Justicia adathoda. (Subbarayappa, 1997). The experimental study was approved under IAEC No: IAEC/XLIV/15/CLBMCP/2014

Plant material and authentication

The raw material Terminalia chebula and Adathodai leaves were purchased from a herbal laboratory/Traditional store. It was authenticated by botanical experts and used for the preparatory process.

Clinical Therapeutics and posology

Fruit rind of 100 (Kadukkai) Terminalia chebula is grinded using fresh leaf juice of (Adathodai) Justicia adathoda and dried well. This procedure is repeated for 14 times. Adult human dose is 1gm thrice a day with honey from the first day of menstrual cycle up to bleeding stops with a regular review for each 7 days. The treatment is given for 3 consecutive cycles.

Animals:

Female and male Wistar albino rats weighing 200 ± 10 g were used. The animals were housed three per cage maximum. For acute toxicity, female were used. Females should be nulliparous and non-pregnant. (Sathasivampillai et al., 2017) Each animal at the commencement of its dosing should be between 8 and 12 weeks old and its weight should fall in an interval within±20 % of the mean weight of the animals. They were fed a normal commercial pellet diet; they were given water ad libitum and maintained under laboratory conditions (temperature 22-24°C, relative humidity 60-70%). The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care. (Ghane et al., 2018).

Acclimatization procedure

The acute and sub acute toxicity animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions. This enhances the study reliability and ensures data stabilization.

Acute toxicity

Acute oral toxicity of kadukkai chooranam is carried out as per the guidelines Organization of Economic Co-operation and Development (OECD) -423 guidelines after the animal ethical clearance from Institutional Animal Ethics Committee. Acute toxicity is involved in estimation of LD$_{50}$ (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals).

MATERIALS AND METHODS

Kadukkai chooranam was taken from the literature Agathiyar attavanai vaagadam used to treat Dysfunctional Uterine Bleeding (Menorrhagia).
The albino mice are fasted over night and provided only water, after which the Kadukkai chooranam is administered by gastric intubations to the relevant group of animals orally at the dose of ranges from 5 mg/g to 200mg/kg body weight.(Mikulski et al., 2017)

The animals are then observed for 14 days and maintained with normal food. A mortality rate of 2 or 3 animals in 14 days is recorded and the dose is said to be toxic dose. However, if mortality is not observed, the procedure is repeated for further higher doses such as 200 and 1,000 mg.kg$^{-1}$ body weight.

Toxic symptoms are observed for 72 hrs including behavioral changes, locomotion, convulsions and mortality.

### RESULTS AND DISCUSSION

#### Acute toxicity

<table>
<thead>
<tr>
<th>Group</th>
<th>Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>Normal</td>
</tr>
<tr>
<td>Assessments of posture</td>
<td>Normal</td>
</tr>
<tr>
<td>Signs of Convulsion</td>
<td>Absence (-)</td>
</tr>
<tr>
<td>Limb paralysis</td>
<td></td>
</tr>
<tr>
<td>Body tone</td>
<td>Normal</td>
</tr>
<tr>
<td>Lacrimation</td>
<td>Absence</td>
</tr>
<tr>
<td>Salivation</td>
<td>Absence</td>
</tr>
<tr>
<td>Change in skin color</td>
<td>No significant colour change</td>
</tr>
<tr>
<td>Piloerection</td>
<td>Normal</td>
</tr>
<tr>
<td>Defecation</td>
<td>Normal</td>
</tr>
<tr>
<td>Sensitivity response</td>
<td>Normal</td>
</tr>
<tr>
<td>Locomotion</td>
<td>Normal</td>
</tr>
<tr>
<td>Muscle gripness</td>
<td>Normal</td>
</tr>
<tr>
<td>Rearing</td>
<td>Mild</td>
</tr>
<tr>
<td>Urination</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Sub-acute toxicity study**

This experiment evaluates the toxicity potential of Kadukkai chooranim. Male and female Wistar rats weighing 200 ± 10 g are used for the present study. The animals are divided into four groups of six animals each (Control, vehicle control, 100mg/kg, 200mg/kg). The dose of the preparation is calculated based on the body weight of the animal.(Baars and Hamre, 2017).

**Experimental procedure**

The dose selected for the sub acute toxicity study was 100mg, 200mg/kg of Kadukkai Chooranam. All the animals were free of intoxicating signs throughout the dosing period of 28 days.
Sub-acute toxicity
Physical observations
No physical changes were observed throughout the dosing period. No mortality was observed during the whole experiment. No abnormal deviations were observed.

Laboratorial/Hematological observations
No significant changes were observed in the values of different parameters studied when compared with controls and values obtained were within normal biological and laboratory limits. There was no significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), Erythrocyte sedimentation rate (ESR) in all the treated groups as compared to respective control groups.

Systemic organ observations
The weights of organs recorded did not show any significant differences in the treatment and the control group indicating that Kadukkai chooranam was not toxic to kidney, liver and spleen.

Histopathology:
Histopathology studies were carried out on liver, kidney and spleen and recorded. Blood samples for hematological and blood chemical analyses were taken from common carotid artery. All rats were sacrificed after the blood collection. The internal organs and some tissues were observed for gross lesions. All tissues were preserved in 10% neutral buffered formaldehyde solution for histopathological examination.

Figure 1. White blood cell count difference between the therapeutic doses

![Graph showing white blood cell count difference between 100mg/kg and 200mg/kg doses]

Table 2. Behavioural sign observation of acute toxicity study

| Dose mg/kg | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|-----------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| 1000      | +  | -  | -  | -  | -  | +  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | +  |

Figure 2. Platelet observation between two doses

![Platelet observation between two doses](image)

Figure 3. Differential count variation between two dosage groups

![Differential count variation between two dosage groups](image)

Hematology parameters of Kadukkai chooranam 100 mg/kg:
- RBC: 6.7 millions/cumm
- HB: 15.5 gms%
- PCV: 45%
- MCV: 48.5 fL
- MCH: 18.5 pg
- MCHC: 29.3 Grams/dl

LIPID PROFILE
- T. Cholesterol: 111 mg/dl
- Triglycerides: 34 mg/dl
- HDL: 23 mg/dl
- LDL: 65.2 mg/dl
- VLDL: 15.8 mg/dl
- Ratio 1(T.CHO/HDL): 4.44
- Ratio 2(LDL/HDL): 2.89

When comparing the white blood cell range between the two doses, in higher dose of 200mg/kg the level was reduced to 2000cell/cumm. The exponential curve shows the declining deviation for this component (figure 1).
DISCUSSION

The acute toxicity study of *Kadukkai chooranam* was carried out as per OECD-423 guidelines, no mortality was observed in both the animals of control group as well as animals treated with a maximum dose of 1000 mg.kg\(^{-1}\). Hence, 1/10\(^{th}\) of 1000 mg.kg\(^{-1}\) i.e. 100 mg.kg\(^{-1}\) of dose was selected as a minimum dose and 200 mg.kg\(^{-1}\) were confined as the higher limit dose for sub-Chronic toxicity study. The results of sub-chronic toxicity study show that there was no significant change in animal behavior due to the absence of toxicity. There are no abnormal features observed in histopathological images.

CONCLUSION

These Siddha herbal drugs *Kadukkai chooranam* are considered as safe as no adverse effect on biochemical and hematological parameters and histopathology of kidney, liver, and spleen was observed even after administering these drugs for a long period.

SOURCE OF FUNDING

Nil

CONFLICT OF INTEREST

None declared.

REFERENCES


